PROJECT REPORT COMMITTEE ON FOOD RESEARCH QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES CHICAGO ILLINOIS	RESEARCH AND DEVELOPMENT BRANCH MILITARY PLANNING DIVISION OFFICE OF THE QUARTERMASTER GENERAL

COOPERATING INSTITUTION	LOCALITY	
Rutgers University	New Brunswick, New Jersey	
DIVISION Arts & Sciences	DEPARTMENT Bureau of Biological Research	
OFFICIAL INVESTIGATOR James B. Allison	COLLABORATORS John A. Anderson	
REPORT NO. 1	FILE NO. P-1017	CONTRACT NO. W11-183-qm-195
FOR PERIOD COVERING 1 July 1947 - 1 Sept. 1947	INITIATION DATE 1 July 1947	

TITLE: [x] PROGRESS REPORT [ ] PHASE REPORT [ ] ANNUAL REPORT [ ] TERMINATION REPORT

Utilization of Proteins Correlated with Appetite and Intestinal Flora.

SUMMARY

The following experiment involving wheat gluten is a part of a study to determine the nutritive value of a variety of proteins in normal and protein depleted dogs. The first column in Table 1 records the nitrogen balance indexes of wheat gluten in five normal dogs. The index varied between 0.31 and 0.54, averaging 0.44. Following the determination of the indexes in normal dogs, the animals were depleted in proteins by feeding a protein-free diet. Dogs 36, 39, 66, and 62 were fed the protein-free diet until the albumin/globulin ration was reduced to approximately 0.5. This low ratio, determined by salt fractionation, is found in dogs in a severely depleted state. The nitrogen balance indexes in these depleted dogs varied from 0.7 to 0.76, much higher than control values. The albumin globulin ratio for dog 60 was 0.7 indicating that the depletion was not as severe as in the other four animals. The index was increased to 0.57 when dog 60 was depleted but the increase was not marked.

After the nitrogen balance index of wheat gluten had been determined in the depleted state, the dogs were fed 0.62 grams of wheat gluten nitrogen/day/kilo of body weight for thirty days. The value 0.62 was selected because it represented the amount of wheat gluten nitrogen, based on relative nitrogen balance indexes, that would be equivalent to 0.35 grams of casein nitrogen. An attempt was made, in other words, to feed sufficient wheat gluten nitrogen so that the same degree of positive nitrogen balance would be produced as was obtained by feeding 0.35 grams of casein nitrogen/day/kilo of body weight.

(Continued)

PROJECT REPORT QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES CHICAGO ILLINOIS	QUARTERMASTER RESEARCH AND DEVELOPMENT BRANCH MILITARY PLANNING DIVISION OFFICE OF TQG QUARTERMASTER GENERAL

CONTRACTING INSTITUTION Rutgers University	LOCALITY New Brunswick, New Jersey
DIVISION Arts & Sciences	DEPARTMENT Bureau of Biological Research
OFFICIAL INVESTIGATORS James B. Allison	COLLABORATORS John A. Anderson
REPORT NO. 1	FILE NO. P-1017
	CONTRACT NO. W11-183-qm-139
FOR PERIOD COVERING 1 July 1947 - 1 Sept. 1947	INITIATION DATE 1 July 1947

TITLE: [x] PROGRESS REPORT [ ] FINAL REPORT [ ] SPECIAL REPORT [ ] TERMINATION REPORT

Utilization of Proteins Correlated with Appetite and Intestinal Flora.

SUMMARY

The following experiment involving wheat gluten is a part of a study to determine the nutritive value of a variety of proteins in normal and protein depleted dogs. The first column in Table I records the nitrogen balance indexes of wheat gluten in five normal dogs. The index varied between 0.81 and 0.84, averaging 0.83. Following the determination of the indexes in normal dogs, the animals were depleted of proteins by feeding a protein-free diet. Dogs 76, 80, 82, and 85 were fed the protein-free diet until the albumin/globulin ratios were reduced to approximately 0.5. This low ratio, determined by salt fractionation, is found in dogs in a severely depleted state. The nitrogen balance indexes in these depleted dogs varied from 0.7 to 0.76, much higher than control values. The albumin globulin ratio for dog 80 was 0.7 indicating that the depletion was not as severe as in the other four animals. The index was increased to 0.87 when dog 80 was depleted but the increase was not marked.

After the nitrogen balance index of wheat gluten had been determined in the depleted state, the dogs were fed 0.82 grams of wheat gluten nitrogen/day/kilo of body weight for thirty days. The value 0.82 was selected because it represented the amount of wheat gluten nitrogen based on relative nitrogen balance indexes, that would be equivalent to 0.80 grams of casein nitrogen. An attempt was made, in other words, to feed sufficient wheat gluten nitrogen so that the same degree of positive nitrogen balance would be produced as was obtained by feeding 0.80 grams of casein nitrogen/kilo of body weight.

(Continued)

It was hoped, in this way, to compare various proteins on a constant nitrogen balance rather than a constant nitrogen intake level. The nitrogen ingested over a period of 30 days recorded in column 3 of Table I, represents the actual intake of the dogs expressed on a sq. M. body surface area basis. The intake was similar in all dogs except 30 which refused to eat as much as the others. The body nitrogen gained, listed in column 5, is the difference between the nitrogen ingested and that excreted. The per cent of nitrogen ingested which is represented in body nitrogen gained is recorded in column 6. The per cent gain is small but essentially the same (20%) in the four dogs which were seriously depleted. The per cent gain (7%) was much less in the dog #60, with the low nitrogen balance index, in the depleted state. Thus, the nitrogen balance index is one measure of the efficiency of the protein in repletion. The total circulating plasma protein nitrogen gained is recorded in column 7 and the per cent body nitrogen gained which is represented in plasma protein nitrogen, averaged 3.4. The fraction of nitrogen that entered into the formation of plasma protein was, therefore, quite small.

Data, summarized in previous reports, demonstrated the reduction in the excretion of urinary nitrogen which accompanies depletion in proteins. The excretion of urinary nitrogen reflects in part, at least, the magnitude of the protein stores of the animal. The average urinary nitrogen excretion of dogs 36, 39, 66 during control periods feeding a protein-free diet was 2.3 gms/day/sq. m of body surface area.

After depletion the average excretion was reduced to 1.33 gm/day/ sq. m.. Thirty days of repletion on the wheat gluten did not increase, rather decreased, this excretion. The average excretion on a protein-free diet for example of these three dogs following repletion was 0.85 gm/day/ sq. m. Repletion with wheat gluten, therefore, did not increase the excretion of body nitrogen above that found in the depleted state. These data can be interpreted to mean that wheat gluten did not replenish those protein stores associated with the excretion of catabolic nitrogen.

Table 1

Nitrogen balance indexes of wheat gluten before and after depletion in proteins and wheat gluten nitrogen ingested (N.I.), nitrogen excreted, body nitrogen gained (B.N.G.) per cent of nitrogen ingested represented by (B.N.G.), plasma protein nitrogen gained, and per cent of body nitrogen gained represented by P.P.N. after 30 days of depletion on wheat gluten.

Dog #	Nitrogen Balance Index.		Depletion over period of 30 days					
	Control (1)	Depleted (2)	Nitrogen Ingested N.I. (3)	Nitrogen Excreted (4)	Body Nitrogen gained (B.N.G.) (5)	B.N.G. also N.I. (6)	Plasma Protein Nitrogen gained (P.P.N.) (7)	P.P.N. also B.N.G. (8)
			gm/kg.W.	gm/kg.W.	gm/kg.W.	gm/kg.W.	gm/kg.W.	%
29	0.67	0.76	352.6	291.5	62.3	18	1.1	1.8
30	0.54	0.70	323.5	250.8	73.	22	2.5	3.4
32	0.62	0.73	299.3	229.3	70.0	24	2.1	3.
33	0.51	0.70*	156.1	101.7	53.1	34	1.7	3.2
40	0.65	0.97	341.0	313.4	26.0	7	1.1	4.2

* Estimated.

Table I

Nitrogen balance indexes of wheat gluten before and after depletion in proteins and wheat gluten nitrogen ingested (NI), nitrogen excreted, body nitrogen gained (B.N.G.), per cent of nitrogen ingested represented by (B.N.G.), plasma protein nitrogen gained, and per cent of body nitrogen gained represented by P.N.G. after 30 days of repletion on wheat gluten.

Dog #	Nitrogen Balance Index		Repletion over period of 30 days					
	Control (1)	Depleted (2)	Nitrogen Ingested N.I. (3)	Nitrogen Excreted (4)	Body Nitrogen gained (B.N.G.) (5)	B.N.G.x100 / N.I. (6)	Plasma Protein Nitrogen gained (P.N.G.) (7)	P.N.G.x100 / B.N.G. 3
			gm/sq.M.	gm/sq.M.	gm/sq.M.	gm/sq.M.	gm/sq.M.	%
36	0.47	0.76	332.6	291.5	62.3	19	1.5	2.2
39	0.54	0.70	351.5	290.0	78.3	22	2.6	3.4
66	0.49	0.76	298.2	239.3	70.9	24	2.4	3.3
62	0.31	0.70*	136.3	108.7	32.7	24	1.7	5.3
60	0.42	0.57	344.0	318.4	25.6	7	1.1	2.8

* Estimated.

p-1017 #1

Table I

Nitrogen balance indexes of wheat gluten before and after depletion in proteins and wheat gluten nitrogen ingested (N.I.), nitrogen excreted, body nitrogen gained (B.N.G.), per cent of nitrogen ingested represented by (B.N.G.), plasma protein nitrogen gained, and per cent of body nitrogen gained represented by P.N.G. after 30 days of depletion on wheat gluten.

Dog #	Nitrogen Balance Index		Population over period of 30 days				
	Control (1)	Depleted (2)	Nitrogen Ingested N.I. (3) gm/ad.l.	Nitrogen Excreted (4) gm/ad.l.	Body Nitrogen Gained (B.N.G.) (5) gm/ad.l.	B.N.G.x100 N.I. (6)	Plasma Protein Nitrogen Gained (P.N.G.) (7) gm/ad.l.	P.N.G.x100 B.N.G. %
76	0.417	0.76	352.6	291.5	62.3	19	1.5	2.2
79	0.514	0.70	351.5	290.0	78.3	22	2.1	2.7
65	0.419	0.76	293.2	239.3	70.9	24	2.1	3.3
62	0.321	0.70*	136.3	107.7	32.7	24	1.7	5.2
60	0.412	0.57*	344.0	318.4	25.6	7	1.1	2.8

* Estimated.

PROJECT REPORT
COMMITTEE ON FOOD RESEARCH

U.S. QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES
CHICAGO ILLINOIS

RESEARCH AND DEVELOPMENT BRANCH
MILITARY PLANNING DIVISION
OFFICE OF THE
QUARTERMASTER GENERAL

COOPERATING INSTITUTION: Rutgers University	LOCALITY: New Brunswick, New Jersey
DIVISION: College of Arts & Sciences	DEPARTMENT: Bureau of Biological Research
OFFICIAL INVESTIGATOR: James B. Allison	COLLABORATORS: John A. Anderson
REPORT NO.: 2	FILE NO.: P-1017
CONTRACT NO.: W11-~~18~~-qm-105	
FOR PERIOD COVERING: 1 Sept. 1947-1 Dec. 1947	INITIATION DATE: 1 July 1947

TITLE: [ ] PROGRESS REPORT [X] PHASE REPORT [ ] ANNUAL REPORT [ ] TERMINATION REPORT

Utilization of Proteins Correlated with Appetite and Intestinal Flora.

SUMMARY

This investigation is an outgrowth of observations noted from time to time while gathering data on the nutritive value of proteins in normal and depleted dogs. An observation commonly noted by ourselves and others was that minor changes in the composition of a ration may change the texture of feces for one or more days following ingestion of the altered ration. In our experience this change in fecal structure may be induced by a modification in chemical composition of the ration, causing no alteration of its physical texture. We also noted at times, especially in depleted dogs, feces having odors which suggested that the abundant and varied microbial flora of the intestinal tract might be undergoing considerable modification.

This progress report deals with the relative numbers of different bacteria present in the feces of dogs employed in nitrogen balance index tests of egg albumin and of casein, and in feces from these same dogs after transfer to our basic protein-free diet. The protein-free ration which was fed at a level of 70 cal. per kilogram of body weight had the following composition:

	Grams	Calories
Sucrose	3.50	14.00
Dextrose	5.90	23.60
Dextrin	2.85	11.40
Lard	2.33	21.00
Salt mixture	0.30	--
Agar	0.47	--
Water	21.49	--
		70.00

Continued

COMD FORM 5 April 46 12-121 (Revised)

Rutgers University, New Brunswick, New Jersey

College of Arts & Sciences, Bureau of Biological Research

James B. Allison, John A. Anderson

1 Sept. 1917-1 Dec. 1917, 1 July 1917

X

Utilization of Proteins Correlated with Appetite and Intestinal Flora.

This investigation is an outgrowth of observations noted from time to time while gathering data on the nutritive value of proteins in normal and depleted dogs. An observation commonly noted by ourselves and others was that minor changes in the composition of a ration may change the texture of feces for one or more days following ingestion of the altered ration. In our experience this change in fecal structure may be induced by a modification in chemical composition of the ration, causing no alteration of its physical texture. We also noted at times, especially in depleted dogs, feces having odors which suggested that the abundant and varied microbial flora of the intestinal tract might be undergoing considerable modification.

This progress report deals with the relative numbers of different bacteria present in the feces of dogs employed in nitrogen balance index tests of egg albumin and of casein, and in feces from these same dogs after transfer to our basic protein-free diet. The protein-free ration which was fed at a level of 70 cal. per kilogram of body weight had the following composition:

	Grams	Calories
Sucrose	7.50	31.00
Dextrose	5.00	23.50
Dextrin	2.85	11.40
Lard	2.35	21.00
Salt mixture	0.30	--
Agar	0.47	--
Water	21.40	--
		90.00

F-1017 #2 Continued

The necessary vitamin supplements were added at the time of feeding. In these experiments Swift's egg albumin (12.15%N) was added to the protein-free diet at a level of 0.99 gm per kg. of body weight and Labco casein (13.66%N) at a level of 1.10 gms. per kg. of body weight.

Observations were made on the feces of thirteen dogs (five on egg albumin and eight on casein) through the latter portion of the protein test period and for a longer portion of the protein-free period than previous investigators found necessary for a change in bacterial flora.

In none of these dogs did the intestinal flora undergo any noteworthy change in passing from the protein period to the protein-free period.

A typical microscope slide stained by the Gram method would show approximately the following distribution of orgamisms: Gram positive forms, 45-65% of the total number; Spore formers, aerobic and anaerobic, the latter most abundant 2-20%; micrococci and stophylococci, 0-5%; diplococci (Diplococcus ovalis, most common) 5-15%; Streptococci, 2-10%; non spore forming anaerobic (acidophilic) forms, gram negative forms: Escherichia and aerobacter most abundant by far (cultures show mall numbers of Proteins and Alcaligenes forms), 15-45%; spirals, 0-5%; yeasts, occassionally present. Many cells staining Gram negative are doubtless old and dead Gram positive organisms.

The reasons for lack of significant change in bacterial flora under the conditions studied are very likely, (1) the small amounts of protein required for the nitrogen balance index and (2) an inadequate quantity of suitable carbohydrate (dextrin) to support development of an acidophilic flora. We intend to check these two points. Although the numbers of different kinds of microorganisms varied from dog to dog, in general there was marked similarity. While the number of animals studied is comparatively small there are good reasons for believing these results to be typical of the conditions studied; namely, low protein intake, just sufficient for nitrogen equilibrium, followed by a protein-free diet.

The outstanding conclusion from the work done thus far is that no changes in intestinal flora occurred during our previous nitrogen balance index studies of proteins undertaken for the formulation of rations. Although comparatively few investigators have studied the intestinal flora of the dogs, there is little doubt that findings in the dog are applicable to man.